Germination, Growth Rates, and Electron Microscope Analysis of Tomato Seeds Flown On The LDEF

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ABSTRACT

The tomato seeds were flown in orbit aboard the Long Duration Exposure Facility (LDEF) for nearly six years. During this time, the tomato seeds received an abundant exposure to cosmic radiation and solar wind. Upon the return of the LDEF to earth, the seeds were distributed throughout the United States and 30 foreign countries for analysis. The purpose of the experiment was to determine the long term effect of cosmic rays on living tissue. Our university analysis included germination and growth rates as well as Scanning Electron Microscopy and X-ray analysis of the control as well as Space-exposed tomato seeds.

In analyzing the seeds under the Electron Microscope, usual observations were performed on the nutritional and epidermis layer of the seed. These layers appeared to be more porous in the Space-exposed seeds than on the Earth-based control seeds. This unusual characteristic may explain the increases in the space seeds growth pattern. (Several test results show that the Space-exposed seeds germinate sooner than the Earth-Based seeds. Also, the Space-exposed seeds grew at a faster rate). The porous nutritional region

may allow the seeds to receive necessary nutrients and liquids more readily, thus enabling the plant to grow at a faster rate.

Roots, leaves and stems were cut into small sections and mounted. After sputter coating the specimens with Argon/Gold Palladium Plasma, they were ready to be viewed under the Electron Microscope. Many micrographs were taken. The X-ray analysis displayed possible identifications of calcium, potassium, chlorine, copper, aluminum, silicon, phosphate, carbon, and sometimes sulfur and iron. The highest concentrations were shown in potassium and calcium. The Space-exposed specimens displayed a high concentration of copper and calcium in the two specimens. There was a significantly high concentration of copper in the Earth-based specimens, whereas there was no copper in the Space-exposed specimens.

Introduction

The long-term effect of cosmic environmental condition on the normal growth and development of living plant tissue is a key component in understanding man's capabilities for space colonization. Obtained from NASA were Rutgers California Supreme Tomato seeds (Lycopersicon esculentum, var. commune) that were part of the LDEF (Long Duration Exposure Facility) satellite mission. The seeds were hermetically packaged at Park Seed Co., in Greenwood, South Carolina. A portion of the seeds remained at Park Seed Co., in a controlled climate of 21 C with 20% humidity. On April 6, 1984, the Space Shuttle Challenger placed in orbit an additional portion of seeds on board the LDEF. During the LDEF's orbit, the seeds were kept in a scientifically controlled climate of 14 psi with 15% humidity.

The effective use of Scanning Electron Microscopy, Digital Imaging Processing and X-ray Microanalysis were primary techniques used in the understanding of internal and external structures as well as variations in tissue structure of the Rutgers tomato seeds, while excluding water. Hamly (1932) believed that water exclusion was a property of the outer layers of the coat, that is the suberized walls and caps of the Malpighian cells but, more importantly, he showed that impermeability was lost when the highly stressed cells at the strophiole (lens) separated, thus forming a strophiolar cleft and permitting water entry.

The environmental conditions of space such as cosmic radiation, temperature, constant pressure and humidity are examples of abnormal conditions that cause additional stress on plant and animal tissue. This additional stress is thought to be responsible for creating strophiolar clefts in Space-exposed tomato seeds that are larger in size than those created under Earth-based conditions. The two basic internal layers of the seed which include the embryonic layer (contains the ovary) and the endosperm (stores nutrients for growth) are visibly observed by digitally imaged micrographs, taken with the Scanning Electron Microscope, at very high magnification (micrographs 1 and 2). Moreover, note the relative thickness of the LDEF outer seed coat in comparsion with the control seed outer coat; the outer seed coat is 75% smaller for the same position on the seed.

Potential risks to plant and humans in future Space-based controlled ecological life support systems have not been addressed directly (Norman & Schuerger, 1990). The purpose of this study is to show structural changes, along with qualitative element identification and germination rate variations between Earth-based and Space-exposed tomato plants.

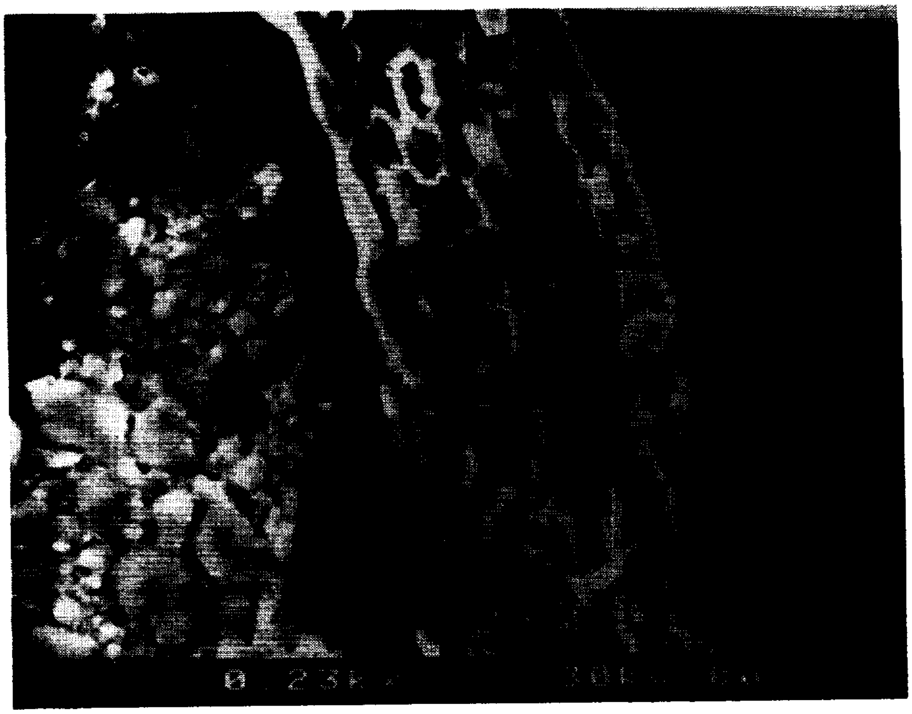


Figure 1. Outer Seed Coat for the Control Seed

Figure 2. Outer Seed Coat for the LDEF Exposed Seed

Table 1 indicates the relative size of the strophiole clefts of the seed coat measured at a magnification of 11.1 KX. The heights of the controls show dramatic increases, while there is a modest increase for the strophiole widths.

TABLE 1.

STROPHIOLE CLEFTS OF THE SEED COAT

BASED ON MEASUREMENTS TAKEN AT A MAGNIFICATION OF 11.1 kx

	SPACE		EARTH	
	HEIGHT	WIDTH	HEIGHT	WIDTH
1.	12.4	4.4	7.1	3.2
2.	11.8	4.2	6.6	3.4
3.	11.4	5.2	6.2	4.1
4.	12.2	5.6	6.3	4.2
5.	13.1	5.1	5.7	4.2
6.	12.6	5.0	5.2	3.8
7.	12.4	4.7	6.1	3.4
8.	10.8	5.1	6.0	3.7
9.	11.9	4.5	5.3	3.1
10.	12.5	4.7	5.5	3.1
MEAN VALUE	12.1	4.8.5	6.0	3.6.1

Table 2

Table 2 shows a relative growth rate analysis over the 3 month period that the sample tomato plants germinated. Note that the group growth rate for A, B, and C of the LDEF exposed seeds have greater heights then the earth based plants heights during the first month of growth. The initial measurements of the heights for the groups A and D show that group A's position had the highest rate of growth when compared to group D's position. Subsequent heights measurements indicate that the earth based plant seeds in comparison with the LDEF show minimal statistical variation in height after the third month.

TABLE II.

GROWTH MEASUREMENTS OF RUTGERS TOMATO PLANTS

DATE RECORDED	MAY 22	JUNE 24	JULY 18
EARTH BASED PLANTS HEIGHT (cm)	2.2 cm	16.4	32.7
SPACED EXPOSED PLANTS HEIGHT (cm)			
GROUP - A	4.41	21.0	30.5
GROUP - B	4.15	17.8	33.7
GROUP - C	3.71	18.5	32.8
GROUP - D	2.7	13.9	28.8

Surface Coat of the Seed

SEM studies have been conducted that demonstrate a unique difference between the LDEF seeds and the control seeds. The control seeds have a wave like surface with minimal surface pores. Moreover, the seed for both the LDEF and control seed have cylindrical structures of some organic nature, but the LDEF exposed seeds have cylindrical structures which are less symmetrical as shown in micrograph Figures 4 and 9. The most fascinating observation is that the LDEF exposed seed have many porous structures which are visible in the center portion and outer edge of the seed. This may explain why the LDEF exposed seeds have faster germination rate than the control seeds.

The seeds may have undergone some type of early germination, while in the LDEF for the 69 month trip. The 3000X micrograph Figure 6 indicates the internal structure of the numerous pores observed using the LDEF seed. In micrograph Figure 3 one can clearly see the pores on the LDEF exposed-seeds. Micrograph Figures 4, 5, 6 indicate increased magnification of the pores region 209X to 3150X magnification. Figure 6, the highest magnification level, shows the organic matter of an individual pore in the LDEF exposed-seeds.

Figures 7, 8, 9 show increased magnification of the control seed whose surface has minimal porous openings. In Figure 9 at 210X magnification the surface shows minimal indication of pores for the control seed.

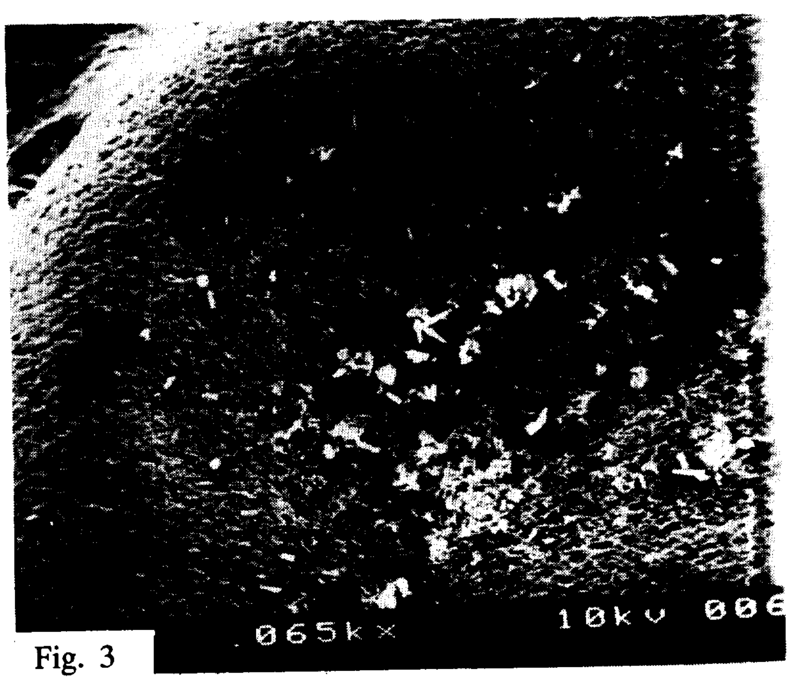


Fig. 3

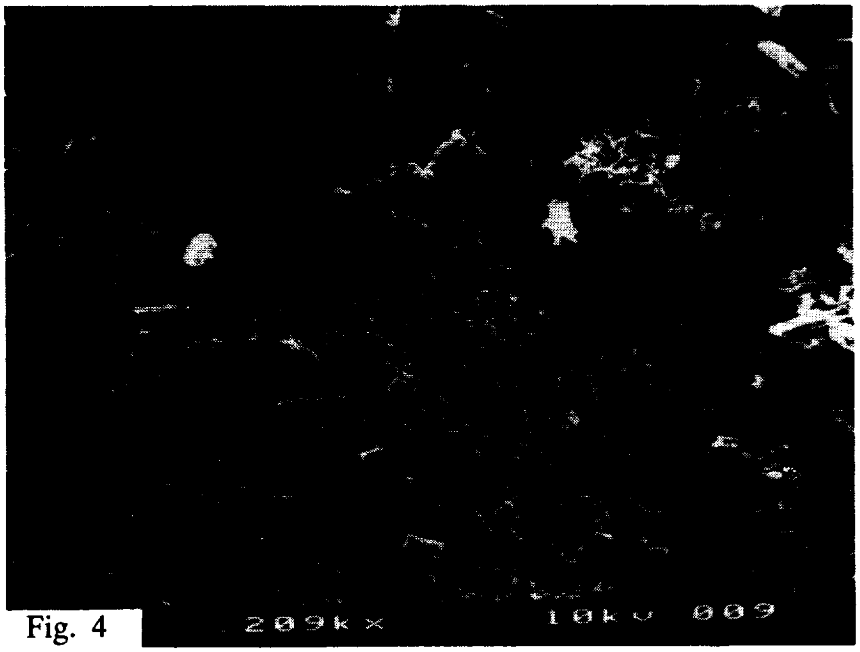


Fig. 4

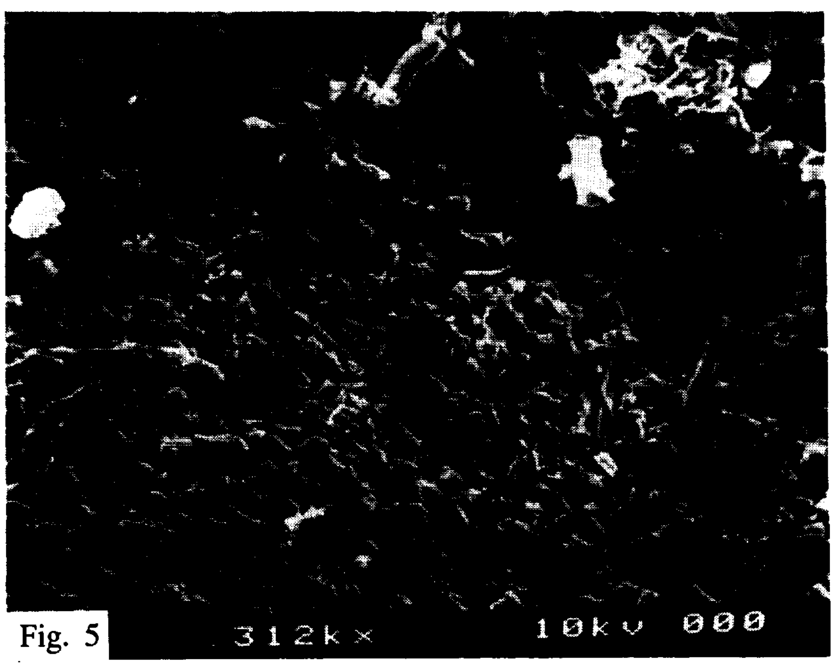


Fig. 5

Fig. 6

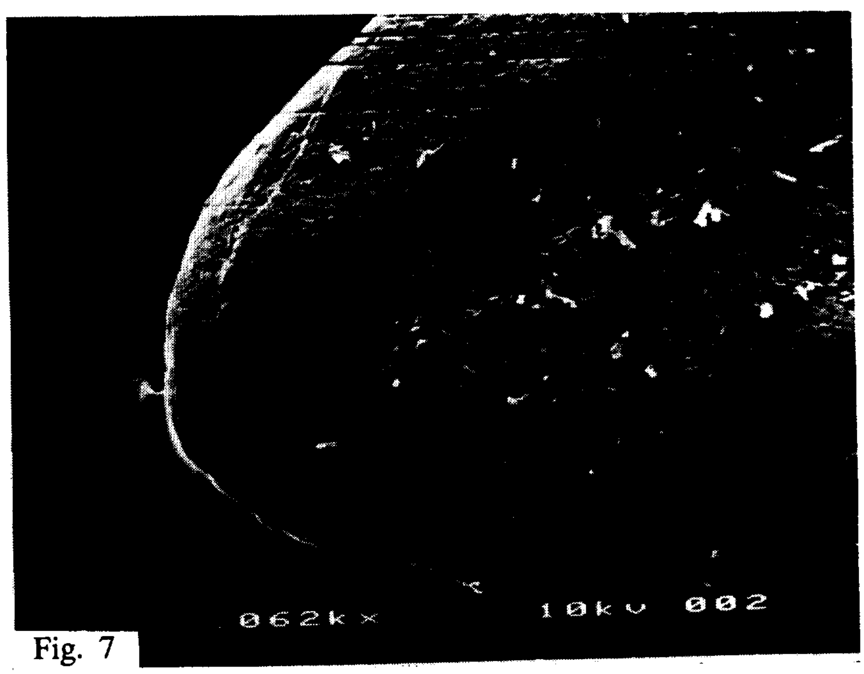


Fig. 7

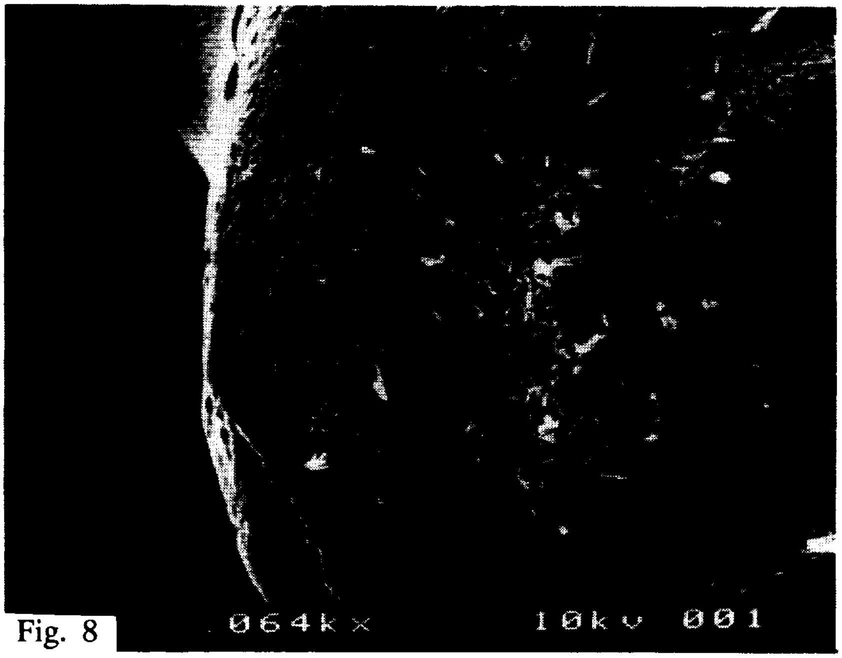


Fig. 8

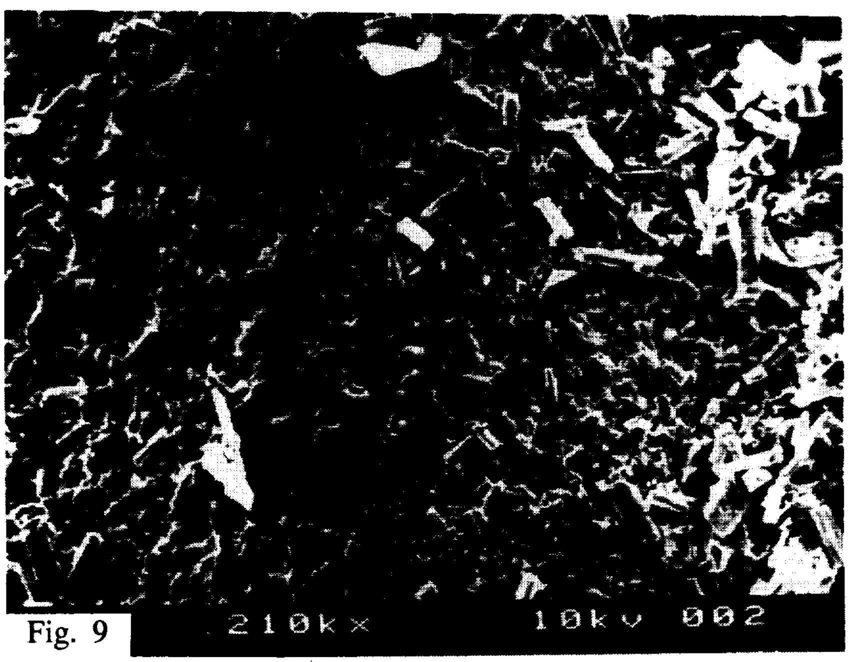


Fig. 9

Comparative Analysis of the Internal Seed Structure

The dramatic difference between the LDEF exposed seed is observed when the seed is opened to expose the tomato plant embryo, the nutritional layer and the outer seed coat. There is a unique and distinct separation between the embryo and the nutritional layer and the nutritional layer and the outer seed coat. The LDEF exposed seeds have a porous material, flaky in nature between the seed embryo, and the nutritional layer. The space between the seed coat and the nutritional layer displays less of the porous filling effect than the inner seed layers. The control or earth bound seeds when opened have distinct absences of an organic material between the embryo and nutritional layer and the nutritional layer and the outer seed coat.

Fig. 10

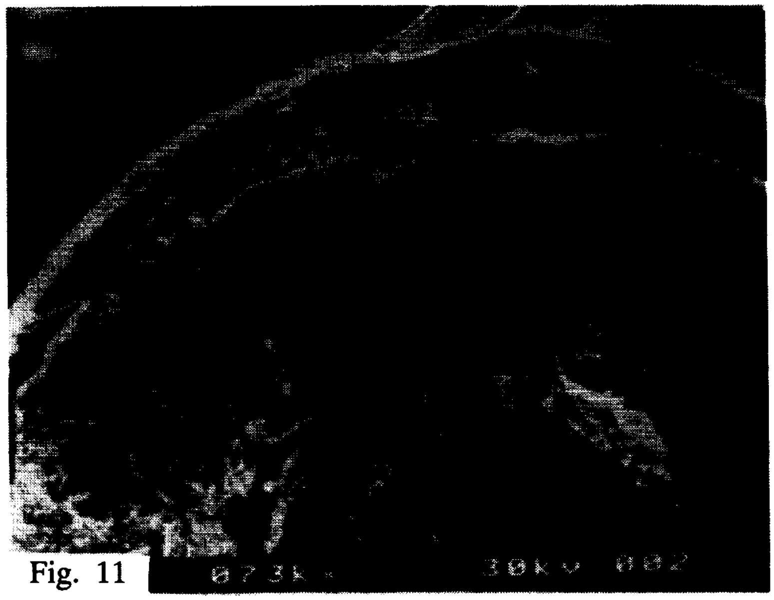


Fig. 11

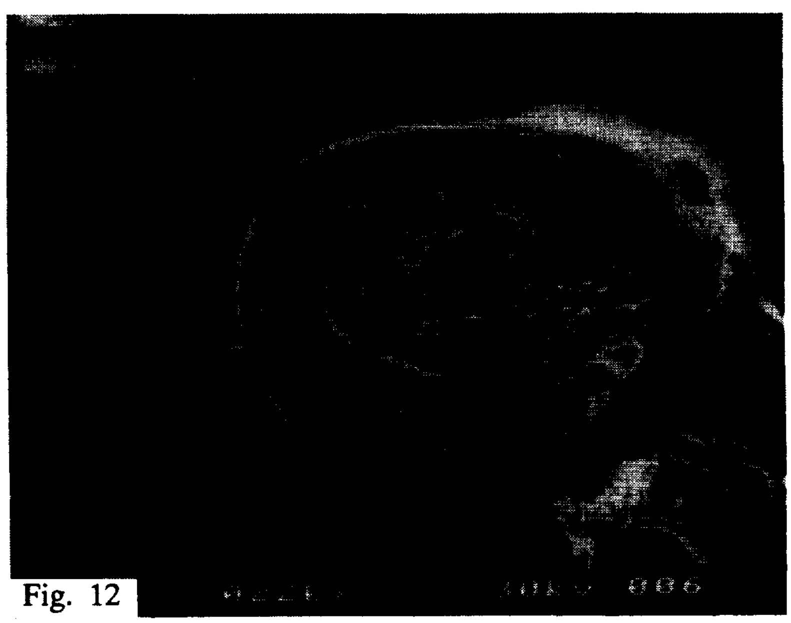


Fig. 12

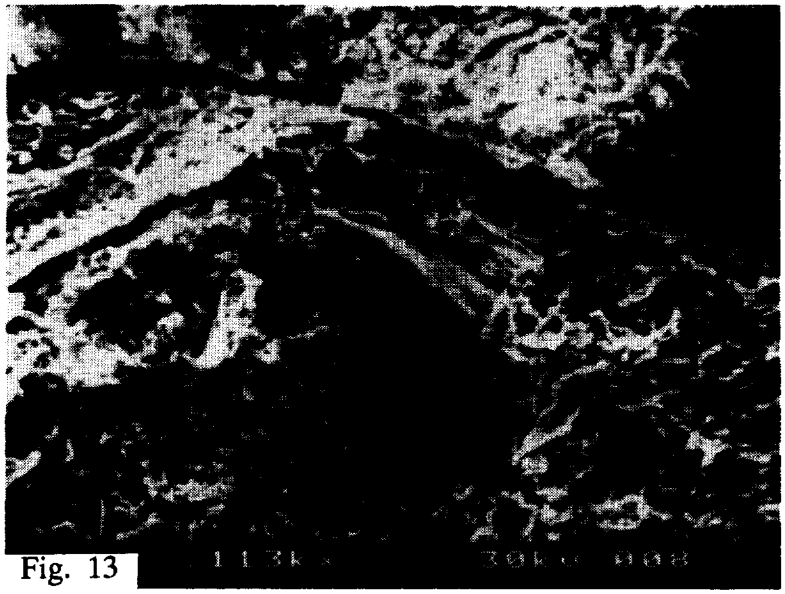


Fig. 13

Root Structure Of Tomato Plant After Germination

The root structure for the LDEF seeds and the control seeds do produce subtle changes in the cluster of materials and other web like structures observed. The earth-control seeds have a higher density of material clusters, stringy web like structures, than the LDEF seeds. An actual quantitative measure requires a study using statistical techniques to demonstrate the validity of the above preposition.

Figure 14. Young Root from LDEF Seed

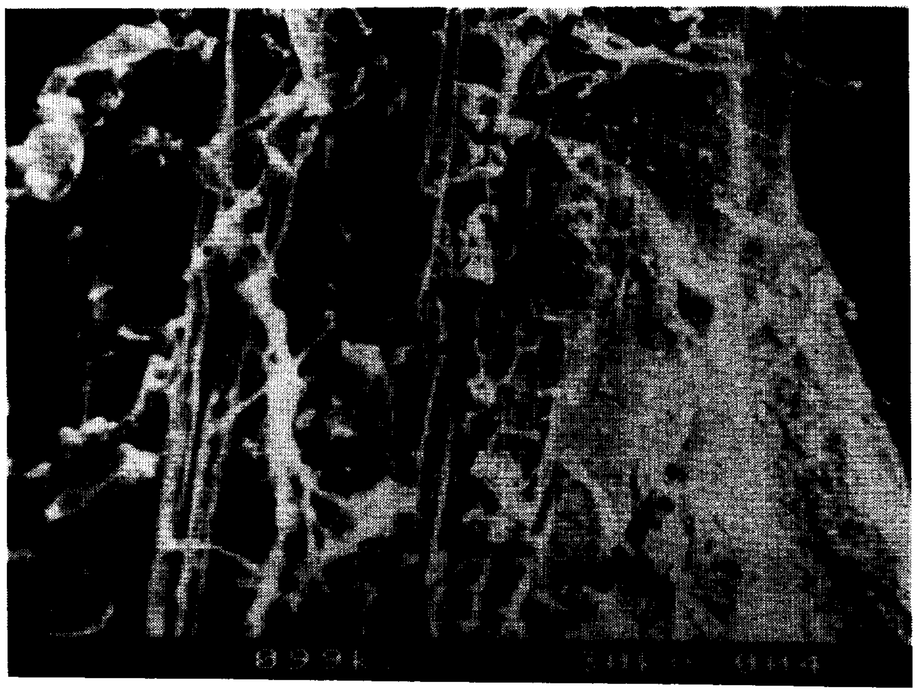


Figure 15. Young Root from Control Seed

Leaf Structure And Calculation Of Comparable Structures

The leaf structure of the LDEF young plant versus the control young plant is remarkable because of the wave like structure that permeates the young leaf surface. The micrograph of the control leaf was taken at 980X, while the Space plant leaf was taken at 300X magnification. The leaves were removed at the same time from the young tomato plant. A calculation of similar structures in the LDEF leaf produces 6 times the length of the control germinated plant leaf structure. This further confirms the increased germination rate of the plant from the LDEF exposed seed.

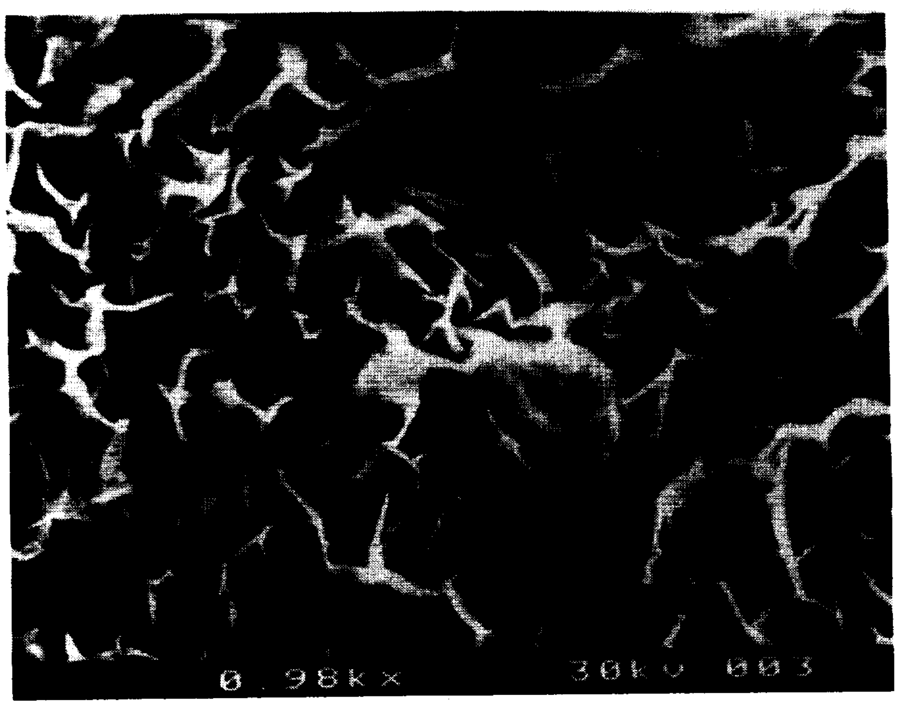


Figure 16. Leaf from Control Seed

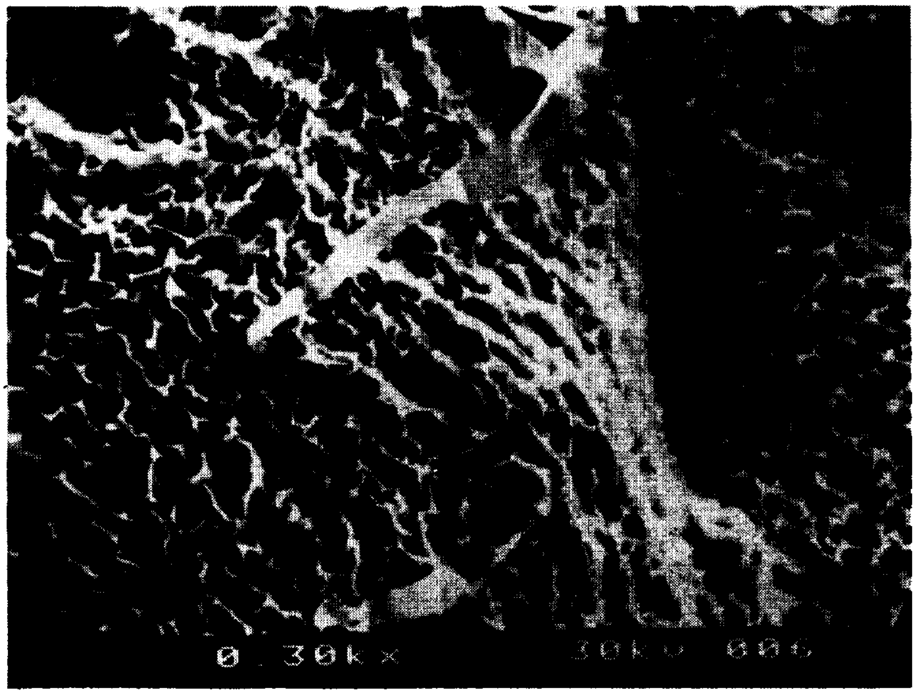


Figure 17. Leaf from LDEF Exposed Seed

The EDS X-ray micro analysis produces a list of eight elements which are common to the space as well as the earth seeds. Aluminum and silver peaks are observed because the SEM mounts were made of aluminum and silver paint used to fasten the seeds to the stub. The peaks associated with carbon, potassium, copper, and calcium have been observed by other researchers including John N. Lott and colleagues. Elements such as chlorine and rhenium cannot be explained except that it may be another elemental peak masking as those elements. The most unique observation is that iron is seen in the controls while the seeds on board LDEF produced substantial peaks of magnesium, phosphorus and sulfur. Other Lott research team reports assert that there are globoid crystals clusters in the embryo region containing P, K and Mg. The question remains why the existence of these elements P, K and Mg in the LDEF exposed seeds, while the element Fe was observed in the controls. This may be an artifact of the particular seed examined.

RESULTS

X-RAY MICROANALYSIS

THE X-RAY MICROANALYSIS RESULTS OF THE ELEMENTAL COMPOSITION OF BOTH EARTH-BASED AND SPACE-EXPOSED RUTGERS TOMATO PLANTS AND SEEDS ARE AS FOLLOWS:

SPACE	EARTH
C	C
Al	Al
Cl	Cl
K	K
Cu	Cu
Si	Si
Ca	Ca
Re	Re
Mg	Fe
P	
S	

Discussion

The Strophiole region of the seed coat is considered to be the region more susceptible to permeability than any other region of the seed coat. The Strophiole clefts (pores of the strophiole region) of the Space-exposed seeds were larger in size than those clefts found in the strophiole region of Earth-based tomato seeds. Thus, allowing the rate of permeability in the Space-exposed seeds, to occur at a high rate than permeability in the Earth-based seed. This is related to the higher germination rates in the Space-exposed seeds. However, during the remainder of plant development, the Earth-based plants sprouted and grew to the approximate size of the Space-exposed plants.

The visual observation of the internal and external structures of the Rutgers tomato seeds showed a greater separation of the seed coat from the endosperm/cotyledon layer of Space-exposed seeds in comparison to the Earth-based seeds.

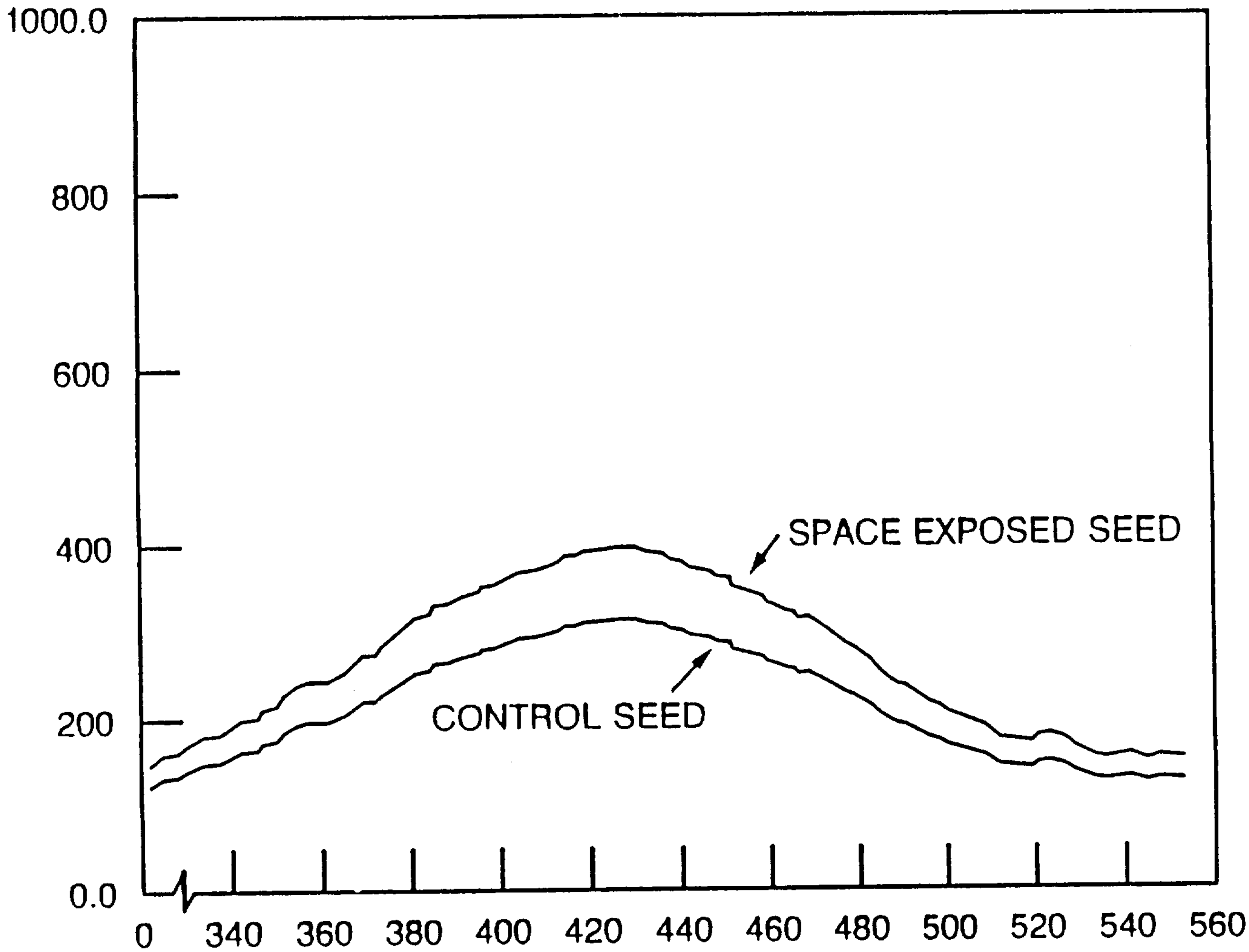
The elemental composition found in the globoid crystals of the tomato seed contain P, K and Mg, but some may also have traces of Ca, Fe and Mn (Spitzer and Lott, 1980). Throughout the embryo in the endosperm, the occasional cells contain Ca in its globoid crystals (Murray, 1984). In this study we found that the elemental composition of both Earth-based and Space-based seeds contained, the elements that were found in previous studies. However, there were additional elements found in this study that had not been cited previously. The relationship of these elements to the growth and development of the seeds in this study has not yet been concluded.

Future research should refine the technique of analyzing the organic material on the seed coat, the germinating root structure, and the leaf and stem structure. It is hoped that higher magnification will be achieved utilizing the Scanning Electron Microscope and ultimately the Scanning Tunneling Microscope and Atomic Force Microscope to look at biological structures of seeds at atomic resolution levels. Micrograph Figure 18 shows an in-depth picture of one of the pores within the Space-exposed seeds surface at approximately 5000X magnification. As a prime example of the microscopic limits using the tomato seeds. Figure 19 shows globoid clusters at magnifications of 1000X and X-ray analysis can reveal its elemental composition with accuracy. Moreover, Fluorescence studies of the LDEF exposed seeds in comparison to the controlled seeds using excitation frequency of 300 nm and emission maximum frequency of 587 nm indicate that the surface of the LDEF seeds have a 30% increase in Fluorescent intensity compared to the control seed

Fluorescence. There is also a slight shift toward the higher wavelength for the control seed which has the reduced intensity.

Figure 18. Isolated Pore in LDEF Exposed Seed

Figure 19. Globoid cluster at 1030X magnification



1000.0
800
600
400
200
0.0
SPACE EXPOSED SEED
CONTROL SEED
0
340
360
380
400
420
440
460
480
500
520
540
560

Figure 20

References

1. Barber, T. A. (1976). In Principal and Techniques of SEM: Biological Application, Vol 5, ed. M.A. Hayat, Van Nostrand Reinhold, New York.

2. Chandler, J.A. (1977). "X-Ray Microanalysis in the Electron Microscopy", In Practical Methods in Electron Microscopy, Vol 5, ed. A.M. Glauert, North-Holland, Amsterdam.

3. Goldstein, Newbury, Echlin, Joy, Foiri, Lifshin (1977). Scanning Electron Microscopy and X-Ray Microanalysis. Plenum Press, New York.

4. Hamly, D.H. (1932) Bot. Gaz. 39, 345-375.

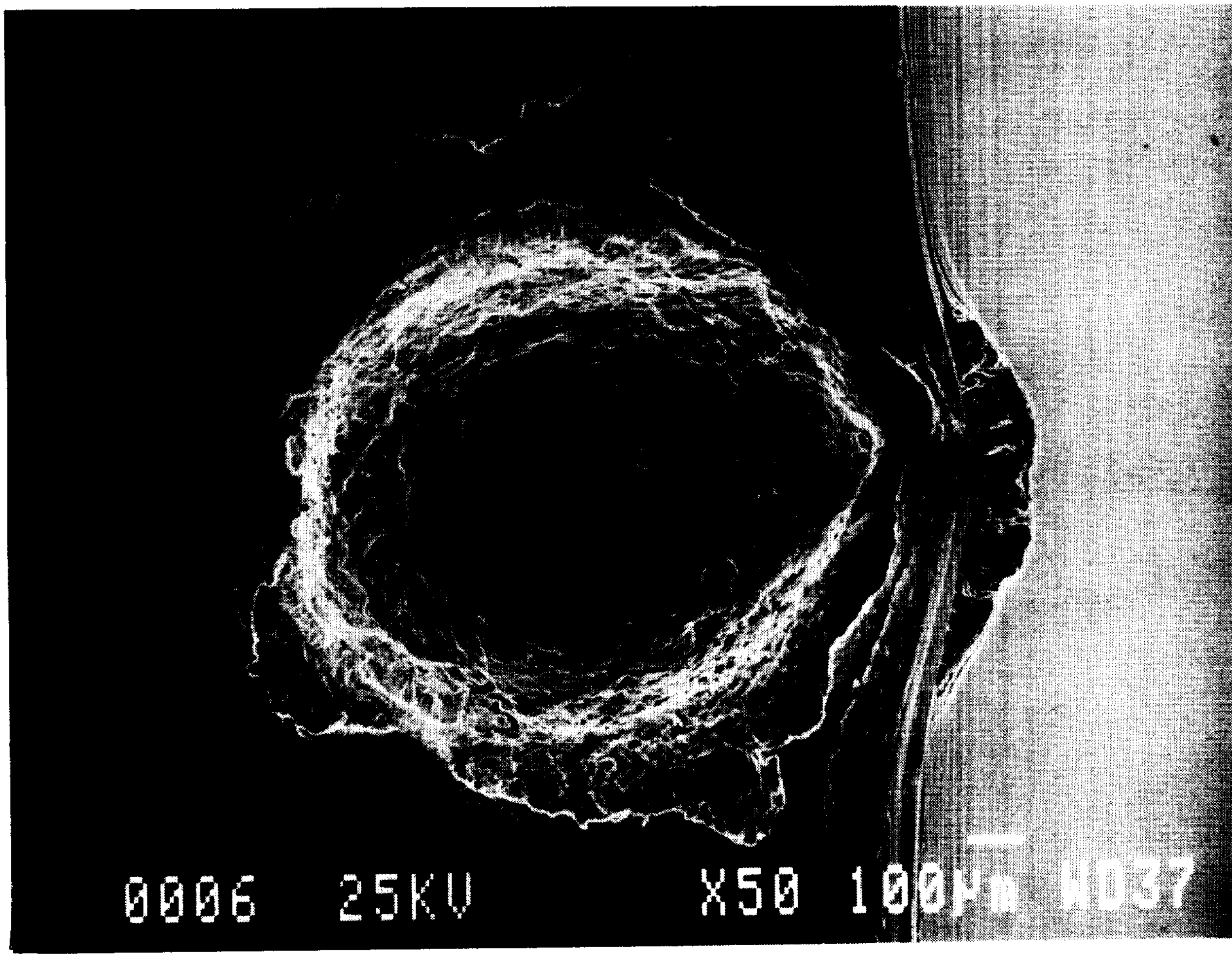
5. Murray, D.R., and Roxburgh, C. Mc C. (1984) J. Sci. Food Agric. 35.

6. Spritzer, E., and Lott, J.N.A. (1980) Can J. Bot. 58. 699-711.

7. Taylor, N. (1989). Taylor Encyclopedia of Gardening Horticulture and Landscape Design 4th edition, Houghton, Mifflin Co. Boston, MA.

8. Wyman , D (1977). Wymans Gardening Encyclopedia, Macmillian Publishing Co., Inc. USA.

SPACE ENVIRONMENTS
Meteoroid and Debris



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